PCT/EP00/02998

CLAIMS

5

10

15

20

25

30

Method for the locus-specific, separate amplification of exon 2, exon 3 and/or exon 4 of HLA-A, HLA-B or HLA-C alleles, making use of at least one primer set wherein:

- for the amplification of exon 2, the reverse primer specifically hybridizes to a locus-specific target sequence in intron 2 of respectively HLA-A, HLA-B or HLA-C;
- for the amplification of exon 3, the forward primer specifically hybridizes to a locus-specific target sequence in intron 2 of respectively HLA-A, HLA-B or HLA-C and/or the reverse primer specifically hybridizes to a locus-specific target sequence in intron 3 of respectively HLA-A, HLA-B or HLA-C;
- for the amplification of exon 4, the forward primer specifically hybridizes to a locus-specific target sequence in intron 3 of respectively HLA-A, HLA-B or HLA-C.
- 2. Method according to claim a further characterized that the locus-specific target sequence is situated at:
 - position 67, 96, 109, 110, 118, 123, 131 or 181 of the HLA-A intron 2
 (Fig. 1) and/or position 32, 50, 62, 73, 83, 86, 118, 130, 150, 501, 525, 561 or 571 of the HLA-A intron 3 (Fig. 4); or
 - position 35 or 170 of the HLA-B intron 2 (Fig. 2) and/or position 42, 46, 65, 68, 96, 438, 502, 524, 547 or 571 of the HLA-B intron 3 (Fig. 5); or
 - position 84, 107 or 142 of the HLA-C intron 2 (Fig. 3) and/or position
 461, 477, 527, 545 or 561 of the HLA-C intron 3 (Fig. 6).
- 3. Method according to claim 2 further characterized that said positions constitute the 3' end of the primer that is used for the amplification of exon 2, exon 3 or exon 4.
- 4. Method according to claim 3 further characterized that the primer is chosen from the following list:
 - for the amplification of exon 2 of HLA-A (table 1):

	1 1	(
	Sub Acoust	5' GATCTCGGACCCGGAGACTGT3' (SEQ ID NO 2)
	9"/	5'GGATCTCGGACCCGGAGACTGT3' (SEQ ID NO 3)
		5'YGGATCTCGGACCCGGAGACTGT3' (SEQ ID NO 4)
	5	5'GYGGATCTCGGACCCGGAGACTGT3' (SEQ ID NO 5)
		5'GGYOGATCTCGGACCCGGAGACTGT3' (SEQ ID NO 6)
		5'GGTCTCGGRGTCCCGCGGCT3' (SEQ ID NO 7)
		5'GGGTCTCGGRGTCCCGCGGCT3' (SEQ ID NO 8)
		5'AGGGTCTCGGRGTCCCGCGGCT3' (SEQ ID NO 9)
	10	5'AAGGGTCTCGGRGTCCCGCGGCT3' (SEQ ID NO 10)
		5'CAAGGGTCTCGGRGTCCCGCGGCT3' (SEQ ID NO 11)
		5'CTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 12)
		5'TCTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 13)
		5'CTCTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 14)
	15	5'CCTCTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 15)
		5'GCCTCTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 16)
		5'GGCCTCTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 17)
-		5'TCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 18)
		5'CTCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 19)
	20	5'CCTCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 20)
		5'GCCTCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 21)
		5'GGCCTCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 22)
		5'GGGCCTCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 23)
		5'CCTGGGCCTCTCCCGGGDCA3' (SEQ ID NO 30)
	25	5'GCCTGGGCCTCTCCCGGGDC 3' (SEQ ID NO 31)
		5'CGCCTGGGCCTCTCCCGGGDCA3' (SEQ ID NO 32)
		5'GCGCCTGGGCCTCTCCCGGGDCA3' (SEQ ID NO 33)
		5'GGCGCCTGGGCCTCTCCCGGGDdA3' (SEQ ID NO 34)
		5'AGGCGCCTGGGCCTCTCCCGGGDCA3' (SEQ ID NO 35)
	30	5'AGGCGCCTGGGCCTCTCCCG3' (SEQ ID NO 36)
		5'AAGGCGCCTGGGCCTCTCCCG3' (SEQ ID NO 37)
		5'WAAGGCGCCTGGGCCTCTCCCG3' (SFQ ID NO 38)
		5'TWAAGGCGCCTGGGCCTCTCCCG3' (SEQ ID NO 39)

5\'ATCTCGGACCCGGAGACTGT3' (SEQ ID NO 1)

5'GTWAAGGCGCCTGGGCCTCTCCCG3' (\$EQ ID NO 40)

WO 00/61795 PCT/EP00/02998 *GGTWAAGGCGCCTGGGCCTCTCCCG3' (SEQ ID NO 41) CCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 42) 5'ACCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 43) 5'AACCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 44) 5 5'AAACCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 45) 5'GAAACCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 46) 5'TGAAACCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 47) 5'YCCVGCCCGACCAACCYGG3' (SEQ ID NO 48) 5'GYCCVGCCCGACCAACCYGG3' (SEQ ID NO 49) 10 5'YGYCCVGCCCGACCAACCYGG3' (SEQ ID NO 50) 5'CYGYCCVGCCCGACCAACCYGG3' (SEQ ID NO 51) 5'CCYGYCCVGCCCGACCAACCYGG3' (SEQ ID NO 52) 5'CCCYGYCCVGCCCGACCAACCYGG3' (SEQ ID NO 53) for the amplification of exon 3 of HLA-A (table 2; table 3): 15 5'CGGACGGCCRGGTSRCCCA3' (SEQ ID NO 54) 5'ACGGACGGCCRGGT\SRCCCA3' (SEQ ID NO 55) 5'CACGGACGGCCRGGT\SRCCCA3' (SEQ ID NO 56) 5'CCACGGACGGCCRGGT\\$RCCCA3' (SEQ ID NO 57) 5'CCCACGGACGGCCRGGT\\$RCCCA3' (SEQ ID NO 58) 20 5'CCCCACGGACGGCCRGGT\RCCCA3' (SEQ ID NO 59) 5'GGTCCGAGATCCRCCCCGAA'3' (SEQ ID NO 60) 5'GGGTCCGAGATCCRCCCCGAA'3' (SEQ ID NO 61) 5'CGGGTCCGAGATCCRCCCCGAA'\(\frac{1}{3}\)' (SEQ ID NO 62) 5'CCGGGTCCGAGATCCRCCCCGAA'3' (SEQ ID NO 63) 25 5'TCCGGGTCCGAGATCCRCCCCGAA'3' (SEQ ID NO 64) 5'CTCCGGGTCCGAGATCCRCCCCGAA'Y' (SEQ ID NO 65) 5'CCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 66) 5'RCCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 67) 5'CRCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 68) 30 5'CCRCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 69) 5'TCCRCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 70) 5'ATCCCCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 71)

5'CCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 72)

CCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 74) 5'CRCCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 75) 5'CCRCCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 76) 5 5'TCCRCCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 77) 5'CGCGGGACYCCGAGACCCTT3' (SEQ ID NO 84) 5'CCGCGGGACYCCGAGACCCTT3' (SEQ ID NO 85) 5'GCCGCGGGACYCCGAGACCCTT3' (SEQ ID NO 86) 5'AGCCGCGGGACYCCGAGACCCTT3' (SEQ ID NO 87) 10 5'AAGCCGCGGGACYCCGAGACCCTT3' (SEQ ID NO 88) 5'GAAGCCGCGGGACYCCGAGACCCTT3' (SEQ ID NO 89) 5'GACYCCGAGACCCTTGDCCC3' (SEQ ID NO 90) 5'GGACYCCGAGACCCTTGDCCC3' (SEQ ID NO 91) 5'GGGACYCCGAGACCCTTGDCCC3' (SEQ ID NO 92) 15 5'CGGGACYCCGAGACCC\TGDCCC3' (SEQ ID NO 93) 5'GCGGGACYCCGAGACCC'TTGDCCC3' (SEQ ID NO 94) 5'CGCGGGACYCCGAGACCCTTGDCCC3' (SEQ ID NO 95) 5'GACCCTTGDCCCGGGAGAGG3' (SEQ ID NO 96) 5'AGACCCTTGDCCCGGGAGAGG3' (SEQ ID NO 97) 5'GAGACCCTTGDCCCGGGAGAGG\(\frac{1}{3}\)' (SEQ ID NO 98) 20 5'CGAGACCCTTGDCCCGGGAGAGG3' (SEQ ID NO 99) 5'CCGAGACCCTTGDCCCGGGAGAGG3' (SEQ ID NO 100) 5'YCCGAGACCCTTGDCCCGGGAGAGG3' (SEQ ID NO 101) 5'GTTTAGGCCAAAAATCCCCC3' (SEO ID\NO 102) 5'AGTTTAGGCCAAAAATCCCCC3' (SEQ ID NO 103) 25 5'CAGTTTAGGCCAAAAATCCCCC3' (SEQ ID) NO 104) 5'TCAGTTTAGGCCAAAAATCCCCC3' (SEQ II) NO 105) 5'TTCAGTTTAGGCCAAAAATCCCCC3' (SEQ ID NO 106) 5'TTTCAGTTTAGGCCAAAAATCCCCC3' (SEQ ID NO 107) 5'AGCCCGGGAGATCTAYAGGC3' (SEQ ID NO 150) 30 5'CAGCCCGGGAGATCTAYAGGC3' (SEQ ID NO 1\$1)

5'CCAGCCCGGGAGATCTAYAGGC3' (SEQ ID NO 1\(^2\)2) 5'GCCAGCCCGGGAGATCTAYAGGC3' (SEQ ID NO 1\(^2\)3' 5'GGCCAGCCCGGGAGATCTAYAGGC3' (SEQ ID NO 1\(^2\)4

5\CCCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 73)

'AGGCCAGCCCGGGAGATCTAYAGGC3' (SEQ ID NO 155) CCTTGTGGGAGGCCAG3' (SEQ ID NO 156) CCTCCTTGTGGGAGGCCAG3' (SEQ ID NO 157) 5'TCCCCTCCTTGTGGGAGGCCAG3' (SEQ ID NO 158) 5'CTCCCCTCCTTGTGGGAGGCCAG3' (SEQ ID NO 159) 5'TCTCCCCTCCTTGTGGGAGGCCAG3' (SEQ ID NO 160) 5'GTCTCCCCTCCTTGTGGGAGGCCAG3' (SEQ ID NO 161) 5'CCCAAWTGTCTCCCCTCCTT3' (SEQ ID NO 162) 5'TCCCAAWTGTCTCCCCTCCTT3' (SEQ ID NO 163) 5'GTCCCAAWTGTCTCCCCTCCTT3' (SEQ ID NO 164) 5'GGTCCCAAWTGTCTCCCCTCCTT3' (SEQ ID NO 165) 5'TGGTCCCAAW\(\)GTCTCCCCTCCTT3' (SEQ ID NO 166) 5'TTGGTCCCAAWTGTCTCCCCTCCTT3' (SEQ ID NO 167) 5'CTAGTGTTGGTCCCAAWTGT3' (SEQ ID NO 168) 5'TCTAGTGTTGGTCCCAAWTGT3' (SEQ ID NO 169) 5'TTCTAGTGTTGGTCCQAAWTGT3' (SEQ ID NO 170) 5'ATTCTAGTGTTGGTCCQAAWTGT3' (SEQ ID NO 171) 5'TATTCTAGTGTTGGTCCCAAWTGT3' (SEQ ID NO 172) 5'ATATTCTAGTGTTGGTCCCAAWTGT3' (SEQ ID NO 173) 5'GGGYGATATTCTAGTGTTGG3' (SEQ ID NO 174) 5'AGGGYGATATTCTAGTGTTGG3' (SEQ ID NO 175) 5'GAGGGYGATATTCTAGTGTTGG3' (SEQ ID NO 176) 5'GGAGGGYGATATTCTAGTGTTGG\(\frac{3}{2}\)' (SEQ ID NO 177) 5'GGGAGGGYGATATTCTAGTGTTGG3' (SEQ ID NO 178) 5'AGGGAGGGYGATATTCTAGTGTTGG'X' (SEQ ID NO 179) 5'GGAGGGYGATATTCTAGTGT3' (SEQ ID NO 180) 5'GGGAGGGYGATATTCTAGTGT3' (SEQ ID\NO 181) 5'AGGGAGGGYGATATTCTAGTGT3' (SEQ ID\NO 182) 5'GAGGGAGGGYGATATTCTAGTGT3' (SEQ ID\NO 183) 5'AGAGGGAGGGYGATATTCTAGTGT3' (SEQ ID NO 184) 5'CAGAGGGAGGGYGATATTCTAGTGT3' (SEQ ID NO 185) 5'CCCAGGAGGAKTCCTCTCCC3' (SEQ ID NO 186) 5'ACCCAGGAGGAKTCCTCTCCC3' (SEQ ID NO 187) 5'AACCCAGGAGGAKTCCTCTCCC3' (SEQ ID NO 188)

10

15

20

25

30

5'GAAACCCAGGAGGAKTCCTCTCCC3' (SEQ ID NO 189)
5'GAAACCCAGGAGGAKTCCTCTCCC3' (SEQ ID NO 190)
5'GGAAACCCAGGAGGAKTCCTCTCCC3' (SEQ ID NO 191)
5'AGGATCTGGAAACCCAGGAG3' (SEQ ID NO 192)
5'CAGGATCTGGAAACCCAGGAG3' (SEQ ID NO 193)
5'ACAGGATCTGGAAACCCAGGAG3' (SEQ ID NO 194)
5'TACAGGATCTGGAAACCCAGGAG3' (SEQ ID NO 195)
5'GTACAGGATCTGGAAACCCAGGAG3' (SEQ ID NO 196)
5'GGTACAGGATCTGGAAACCCAGGAG3' (SEQ ID NO 197)
5'TCAGAGTCACTCTCTGGTAC3' (SEQ ID NO 198)
5'CTCAGAGTCACTCTCTGGTAC3' (SEQ ID NO 199)
5'CCTCAGAGTCACTCTCTGGTAC3' (SEQ ID NO 200)
5'ACCTCAGAGTCACTCTCTGGTAC3' (SEQ ID NO 201)
5'AACCTCAGAGTCACTCTCTGGTAC3' (SEQ ID NO 202)
5'GAACCTCAGAGTCACTCTCTGGTAC3' (SEQ ID NO 203)

for the amplification of exon 4 of HLA-A (table 4):

5'TTCTGTGCTCYCTTCCCCAT3' (SEQ ID NO 204)

5'GTTCTGTGCTCYCTTCCCCAT3' (SEQ ID NO 205)

5'GGTTCTGTGCTCYCTTCCCCAT3' (SEQ ID NO 206)

5'GGGTTCTGTGCTCYCTTCCCCAT3' (SEQ ID NO 207)

5'TGGGTTCTGTGCTCYCTTCCCCAT3' (SEQ ID NO 208)

5'CTGGGTTCTGTGCTCYCTTCCCCAT3' (SEQ ID NO 209)

5'GGTGTCCTGTCCATTCTCAA3' (SEQ ID NO 24)

5'RGGTGTCCTGTCCATTCTCAA3' (SEQ ID NO 25)

5'CRGGTGTCCTGTCCATTCTCAA3' (SEQ ID NO 26)

5'CCRGGTGTCCTGTCCATTCTCAA3' (SEQ ID NO 27)

5'CCCRGGTGTCCTGTCCATTCTCAA3' (SEQ ID NO 28)

5'CCCRGGTGTCCTGTCCATTCTCAA3' (SEQ ID NO 28)
5'TCCCRGGTGTCCTGTCCATTCTCAA3' (SEQ ID NO 29)
5'CTGGWGGAGTGTCCCATKAC3' (SEQ ID NO 210)
5'GCTGGWGGAGTGTCCCATKAC3' (SEQ ID NO 211)
5'TGCTGGWGGAGTGTCCCATKAC3' (SEQ ID NO 212)
5'RTGCTGGWGGAGTGTCCCATKAC3' (SEQ ID NO 213)
5'YRTGCTGGWGGAGTGTCCCATKAC3' (SEQ ID NO 214)

10

15

20

25

30

la sela

5'GYRTGCTGGWGGAGTGTCCCATKAC3' (SEQ ID NO 215)

5'GTCCCATKACAGATRCMMAA3' (SEQ ID NO 216)

5'TGTCCCATKACAGATRCMMAA3' (SEQ ID NO 217)

5'GTGTCCCATKACAGATRCMMAA3' (SEQ ID NO 218)

5'AGTGTCCCATKACAGATRCMMAA3' (SEQ ID NO 219)

5'GAGTGTCCCATKACAGATRCMMAA3' (SEQ ID NO 220)

5'GGAGTGTCCCATKACAGATRCMMAA3' (SEQ ID NO 221)

for the amplification of exon 2 of HLA-B (table 5):
 5'ACCCGCGGGGATTTTGGCCTC3' (SEQ ID NO 108)
 5'AACCCGCGGGGATTTTGGCCTC3' (SEQ ID NO 109)
 5'CAACCCGCGGGGATTTTGGCCTC3' (SEQ ID NO 110)
 5'CCAACCCGCGGGGGATTTTGGCCTC3' (SEQ ID NO 111)
 5'MCCAACCCGCGGGGGATTTTGGCCTC3' (SEQ ID NO 112)

5'GMCCAACCGGGGGGATTTTGGCCTC3' (SEQ ID NO 113) 5'YGMCCAACCGGGGGGATTTTGGCCTC3' (SEQ ID NO 314)

- for the amplification of exon 3 of HLA-B (table 6; table 7):

 5'CYGGGGCGSAGGTCACGACT3' (SEQ ID NO 114)

 5'CCYGGGGCGSAGGTCACGACT3' (SEQ ID NO 115)

 5'GCCYGGGGCGSAGGTCACGACT3' (SEQ ID NO 116)

 5'GGCCYGGGGCGSAGGTCACGACT3' (SEQ ID NO 117)

 5'CGGCCYGGGGCGSAGGTCACGACT3' (SEQ ID NO 118)

 5'CCGGCCYGGGGCGSAGGTCACGACT3' (SEQ ID NO 119)

 5'CCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 121)
- 5'CCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 120)
 5'ACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 121)
 5'TACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 122)
 5'TTACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 123)
 5'TTTACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 124)
 5'GTTTACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 222)
 5'CGTTTACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 223)
 5'CGCGTTTACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 223)
 - 5'CGCGTTTACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 224)
 5'CGTKGGAGSCCATCCCCGSC3' (SEQ ID NO 225)

30

	5'CTCGTKGGAGSCCATCCCCGSC3' (SEQ ID NO 226)
	5'CTCGTKGGAGSCCATCCCCGSC3' (SEQ ID NO 227)
	5'TCTCGTKGGAGSCCATCCCCGSC3' (SEQ ID NO 228)
	5'TTCTCGTKGGAGSCCATCCCCGSC3' (SEQ ID NO 229)
5	5'CTTCTCGTKGGAGSCCATCCCCGSC3' (SEQ ID NO 230)
	5'TCTCGTKGGAGSCCATCCCC3' (SEQ ID NO 231)
	5'TTCTCGTKGGAGSCCATCCCC3' (SEQ ID NO 232)
	5'CTTCTCGTKGGAGSCCATCCCC3' (SEQ ID NO 233)
	5'TCTTCTCGTKGGAGSCCATCCCC3' (SEQ ID NO 234)
10	5'YTCTTCTCGTKGGAGSCCATCCCC3' (SEQ ID NO 235)
	5'CYTCTTC CGTKGGAGSCCATCCCC3' (SEQ ID NO 236)
	5'GATCCCATTTTCCTCYTCTT3' (SEQ ID NO 237)
	5'TGATCCCA TTTTCCTCYTCTT3' (SEQ ID NO 238)
	5'CTGATCCCATTTTCCTCYTCTT3' (SEQ ID NO 239)
15	5'GCTGATCCCATTTTCCTCYTCTT3' (SEQ ID NO 240)
	5'CGCTGATCCCATTTTCCTCYTCTT3' (SEQ ID NO 241)
	5'GCGCTGATCCCATTTTCCTCYTCTT3' (SEQ ID NO 242)
	5'GCTGATCCCATTTTCCTCYT3' (SEQ ID NO 243)
	5'CGCTGATCCCATTTTCCTCYT3' (SEQ ID NO 244)
20	5'GCGCTGATCCCAT TTCCTCYT3' (SEQ ID NO 245)
	5'AGCGCTGATCCCAT TTCCTCYT3' (SEQ ID NO 246)
	5'TAGCGCTGATCCCAT(TTCCTCYT3' (SEQ ID NO 247)
	5'CTAGCGCTGATCCCATTTTCCTCYT3' (SEQ ID NO 248)
	5'TCCATTCAAGGGAGGGCGAC3' (SEQ ID NO 249)
25	5'CTCCATTCAAGGGAGGGCGAC3' (SEQ ID NO 250)
	5'TCTCCATTCAAGGGAGGGCGAC3' (SEQ ID NO 251)
	5'TTCTCCATTCAAGGGAGGGCGAC3' (SEQ ID NO 252)
	5'ATTCTCCATTCAAGGGAGGCGAC3' (SEQ ID NO 253)
	S'CATTCTCCATTCA A GGGAGGGCGAC3' (SEO ID NO 254

for the amplification of exon 4 of HLA-B (table 8):

5'AGATTATCCCAGGTGCCTGC3' (SEQ ID NO 255)

5'GAGATTATCCCAGGTGCCTGC3' (SKQ ID NO 256)

5'GGAGATTATCCCAGGTGCCTGC3' (SEQ ID NO 257)

WO 00/61795 PCT/EP00/02998

	(MS) AGGAGATTATCCCAGGTGCCTGC3' (SEQ ID NO 258) 5'TAGGAGATTATCCCAGGTGCCTGC3' (SEQ ID NO 259)
	5'TAGGAGATTATCCCAGGTGCCTGC3' (SEQ ID NO 259)
	5'ATAGGAGATTATCCCAGGTGCCTGC3' (SEQ ID NO 260)
	5'TGTCCTGYCCATTCTCAGKC3' (SEQ ID NO 261)
5	5'GTOTCCTGYCCATTCTCAGKC3' (SEQ ID NO 262)
	5'GGTGTCCTGYCCATTCTCAGKC3' (SEQ ID NO 263)
	5'AGGTGTCCTGYCCATTCTCAGKC3' (SEQ ID NO 264)
	5'CAGGTGTCCTGYCCATTCTCAGKC3' (SEQ ID NO 265)
	5'CCAGGTGTCCTGYCCATTCTCAGKC3' (SEQ ID NO 266)
10	5'TCACATGGGTGGTCCTAGG3' (SEQ ID NO 267)
	5'GTCACATGGGTGGTCCTAGG3' (SEQ ID NO 268)
	5'GGTCACATGGGTGGTCCTAGG3' (SEQ ID NO 269)
	5'TGGTCACATGGGTGGTCCTAGG3' (SEQ ID NO 270)
	5'CTGGTCACATGGGTGGTCCTAGG3' (SEQ ID NO 271)
15	5'KCTGGTCACATGGGTGGTCCTAGG3' (SEQ ID NO 272)
	5'GKCTGGTCACATGGGTGGTCCTAGG3' (SEQ ID NO 273)
	5'TSCCATGARAGATGCMAAGC3' (SEQ ID NO 274)
	5'GTSCCATGARAGATGCMAAGC3' (SEQ ID NO 275)
	5'TGTSCCATGARAGATGCMAAGC3' (SEQ ID NO 276)
20	5'GTGTSCCATGARAGATGCMAAGC3' (SEQ ID NO 277)
	5'GGTGTSCCATGARAGATGCMAAGC3' (SEQ ID NO 278)
	5'GGGTGTSCCATGARAGAGC3' (SEQ ID NO 279)
	5'GWAWTTTCTGACTCTTCCCA3' (SEQ ID NO 280)
	5'TGWAWTTTCTGACTCTTCCCA3' (SEQ ID NO 281)
25	5'CTGWAWTTTCTGACTCTTCCCA3' (SEQ ID NO 282)
	5'CCTGWAWTTTCTGACTC/ITCCCA3' (SEQ ID NO 283)
	5'GCCTGWAWTTTCTGACTGTTCCCA3' (SEQ ID NO 284)
	5'CGCCTGWAWTTTCTGACTCTTCCCA3' (SEQ ID NO 285)
30	- for the amplification of exon 2 of HLA-C (table 9):
	5'GTCGAGGGTCTGGGCGGGTT3 (SEQ ID NO 126)
	5'GGTCGAGGGTCTGGGCGGGTT3' (SEQ ID NO 127)
	5'CGGTCGAGGGTCTGGGCGGGTT3 (SEQ ID NO 128)
	5'CCGGTCGAGGGTCTGGGCGGGTT3' (SEQ ID NO 129)

10

15

20

25

30

WO 00/61795 PCT/EP00/02998

5'YCCGGTCGAGGGTCTGGGCGGGTT3' (SEQ ID NO 130)
5'CYCCGGTCGAGGGTCTGGGCGGGTT3' (SEQ ID NO 131)

- for the amplification of exon 3 of HLA-C (table 10):

5'CGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 132)

5'TCGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 133)

5'GTCGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 134)

5'GGTCGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 135)

5'GGGTCGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 136)

5'CGGGTCGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 137)

5'CGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 138)

5'TCGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 140)

5'CCTCGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 141)

5'CCTCGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 142)

5'ACCCTCGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 143)

for the amplification of exon 4 of HLA-C (table 11):

5'GTGCCTGTGTCCAGGCTGGC3' (SEQ ID NO 286) 5'GGTGCCTGTGTCCAGGCTGGC3' (SEQ ID NO 287)

5'AGGTGCCTGTGTCCAGGCTGGC3' (SEQ ID NO 288)

5'CAGGTGCCTGTGTCCAGGCTGGC3' (SEQ ID NO 289)

5'CCAGGTGCCTGTGTCCAGGCTGGC3' (SEQ ID NO 290)

5'CCCAGGTGCCTGTGTCCAGGCTGGC3' (SEQ ID NO 291)

5'TGGCGTCTGGGTTCTGTGCC3' (SEQ ID NO 292)

5'CTGGCGTCTGGGTTCTGTGCC3' (SEQ ID NO 293)

5'GCTGGCGTCTGGGTTCTGTGCC3' (SEQ ID NO 294)

5'GGCTGGCGTCTGGGGTTCTGTGCC3' (SEQ ID NO 295)

5'AGGCTGGCGTCTGGGGTTCTGTGCC3' (SEQ ID NO 296)

5'CAGGCTGGCGTCTGGGGTTCTGTGCC3' (SEQ ID NO 297)

5'CTCAGGATRGTCACATGGSC3' (SEQ ID NO 298)

5'TCTCAGGATRGTCACATGGSC3' (SEQ ID NO 299)

5'TTCTCAGGATRGTCACATGGSC3' (SEQ ID NO 300)

5'RTTCTCAGGATRGTCACATGGSC3' (SEQ ID NO 301)

ACRTTCTCAGGATRGTCACATGGSC3' (SEQ ID NO 302) CRTTCTCAGGATRGTCACATGGSC3' (SEQ ID NO 303) 5'GGGCTGTTGGAGTGTCGCAA3' (SEQ ID NO 78) 5'GGCTGTTGGAGTGTCGCAA3' (SEQ ID NO 79) 5 5'GGGCGCTGTTGGAGTGTCGCAA3' (SEQ ID NO 80) 5'TGGGCGCTGTTGGAGTGTCGCAA3' (SEQ ID NO 81) 5'ATGGGCGCTGTTGGAGTGTCGCAA3' (SEQ ID NO 82) 5'CATGGGCGCTGTTGGAGTGTCGCAA3' (SEQ ID NO 83) 5'SCAAGAGAGAGAWRCAAAGTGT3' (SEQ ID NO 304) 10 5'CSCAAGAGAGAWRCAAAGTGT3' (SEQ ID NO 305) 5'TCSCAAGAGAGAWRCAAAGTGT3' (SEQ ID NO 306) 5'GTCSCAAGAGAGAWRCAAAGTGT3' (SEQ ID NO 307) 5'TGTCSCAAGAGAGAWRCAAAGTGT3' (SEQ ID NO 308) 5'GTGTCSCAAGAGAGAWRCAAAGTGT3' (SEQ ID NO 309)

15

- 5. Method according to any of claims 1 to 4 further characterized that the amplification of exon 2 is carried out with at least one of the following forward primers:
 - for HLA-A: 5APBio: B-TTCTCCCCAGACGCCGAGGATGGCC (SEQ ID NO 144); or
 - for HLA-B: IBPin1: B-GGGAGGAGCGAGGGGACCSCAG (SEQ ID NO 145); or
 - for HLA-C: 5CIN1: B-AGCGAGGGGCCCGGCGGA (SEQ ID NO 146).

25

30

20

- 6. Method according to any of claims 1 to 5 further characterized that the amplification of exon 4 is carried out with at least one of the following reverse primers:
 - for HLA-A: 3ex4APbio: B-TTGGGCAGACCCTCATGCTGC (SEQ ID NO 311); or
 - for HLA-B: 3ex4IBbio: B-TCGGCAGCCCCTCATGCTGT (SEQ ID NO 312); or

15

20

25

for HLA-C: 3ex4ICbio: B-CATCTCAGGGTGMRGGGCTT (SEQ ID NO

- Method according to any of claims 1 to 6 further characterized that:
- 5 the amplification of exon 2 is carried out with at least one of the following primers sets:
 - for HLA-A: 5APbio (B-TTCTCCCCAGACGCCGAGGATGGCC; SEQ ID NO 144) and 3ex2Apbio (B-ATCTCGGACCCGGAGACTGT; SEQ ID NO 1);
 - for HLA-B: IBPin1 (B-GGGAGGAGCGAGGGGACCSCAG; SEQ ID NO 145) and IB3Pin2bio (B-

AACCCGCGGGGATTTTGGCCTC; SEQ ID NO 109);

- for HLA-C: 5CIN1 (B-AGCGAGGGGCCCGCCCGGCGA; SEQ ID NO 146) and IC3Pin2bio (B-GGTCGAGGGTCTGGGCGGGTT; SEQ ID NO 127);
- the amplification of exon 3 is carried out with at least one of the following primer sets:
 - for HLA-A: 5ex3APblo (B-CAGTTTAGGCCAAAAATCCCCC; SEO ID NO 104) and 3ex3APbio (B-CCCTCCTTGTGGGAGGCCAG; SEQ ID NO 156);
 - for HLA-B: IB5Pin 2bio (B-CGCGTTTACCCGGTTTCATTTTCAGTTG; SEQ ID NO 224) and IB3Pin3bio (B-TCTTCTCGTKGGAGSCCATCCCC; SEQ ID NO 234);
 - for HLA-C: IC5Pin2bio (B-TCGRCCGGRGAGAGCCCCAGT; SEQ ID NO 139) and 3CIN (B-GGAGATGGGGAAGGCTCCCCACT; SEQ ID NO 149);
- the amplification of exon 4 is carried out with at least one of the following primer sets:
- for HLA-A: 5ex4APbio (B-GTTCTGTGCTCYCTTCCCCAT; SEQ ID 30 NO 205) and 3ex4APbio (B-TTGGGCAGACCCTCATGCTOC; SEQ ID NO 311);

Sub Alcondo

5

10

15

for ALA-B: 5ex4IBbio (B-TCACATGGGTGGTCCTAGG; SEQ ID NO 267) and 3ex4IBbio (B-

TCGGCAGCCCCTCATGCTGT; SEQ ID NO 312);

for HLA-C: 5ex4ICbio (B-TCTCAGGATRGTCACATGGSC; SEQ ID

NO 299) and 3ex4ICbio (B-

CATCTCAGGGTGMRGGGCTT; SEQ ID NO 313).

- 8. Method according to any of claims 1 to 7 further characterized that both exon 2 and exon 3 of HLA-A, HLA-B or HLA-C are amplified by use of a multiplex primer mix containing at least one primer pair for the amplification of exon 2 and at least one primer pair for the amplification of exon 3.
- 9. Method according to any of claims 1 to 7 further characterized that all three exons, exon 2, exon 3 and exon 4, of HLA-A, HLA-B or HLA-C are amplified by use of a multiplex primer mix containing at least one primer pair for the amplification of exon 2, at least one primer pair for the amplification of exon 3 and at least one primer pair for the amplification of exon 4.
- 10. A primer as defined by any of claims 1 to 7, for use in the amplification of exon 2
 20 of HLA-A, HLA-B or HLA-C alleles.
 - 11. A primer as defined by any of claims 1 to 7, for use in the amplification of exon 3 of HLA-A, HLA-B or HLA-C alleles.
- 25 12. A primer as defined by any of claims 1 to 7, for use in the amplification of exon 4 of HLA-A, HLA-B or HLA-C alleles.
- 13. A primer set consisting of a combination of a forward and a reverse primer as defined in any of claims 1 to 7, for use in the amplification of exon 2 of HLA-A,
 30 HLA-B or HLA-C alleles.

Gub A' conta

A primer set consisting of a combination of a forward and a reverse primer as defined in any of claims 1 to 7, for use in the amplification of exon 3 of HLA-A, HLA-B or HLA-C alleles.

- 5 15. A primer set consisting of a combination of a forward and a reverse primer as defined in any of claims 1 to 7, for use in the amplification of exon 4 of HLA-A, HLA-B or HIA-C alleles.
- 16. A multiplex primer mix containing at least one primer set according to claim 13 for the amplification of exon 2 and one primer set according to claim 14 for amplification of exon 3.
- 17. A multiplex primer mix containing at least one primer set according to claim 13 for the amplification of exon 2, one primer set according to claim 14 for amplification of exon 3 and one primer set according to claim 15 for the amplification of exon 4.
 - 18. Method for the typing or subtyping of one or more HLA-A, HLA-B or HLA-C alleles in a sample comprising the following steps:
 - (i) if needed, release, isolation and/or concentration of the nucleic acids present in said sample;
 - (ii) amplification of the nucleic acids according to any of claims 1 to 9;
 - (iii) typing of the specific HLA-A, HLA-B or HLA-C allele present in said sample.

25

20

- 19. Method according to claim 18 further characterized that the typing step is carried out by hybridization with one or more suitable probes.
- 20. A diagnostic kit for the typing or subtyping of one or more HLA-A, HLA-B or HLA-C alleles in a sample comprising the following components:
 - (i) when appropriate, a means for releasing, isolating or concentrating the nucleic acids present in said sample;
 - (ii) a primer set or a primer mix according to any of claims 13 to 17;

10

- (iii) a means for the typing of the specific HLA-A, HLA-B or HLA-C allele present in said sample.
- 21. A line probe assay for the typing or subtyping of one or more HLA-A, HLA-B or HLA-C alleles in a sample comprising the following components:
 - (i) when appropriate, a means for releasing, isolating or concentrating the nucleic acids present in said sample;
 - (ii) a primer pair or a primer mix according to any of claims 13 to 17;
 - (iii) at least one probe that specifically hybridizes with exon 2, exon 3 or exon 4 of HLA-A, HLA-B or HLA-C, fixed to a solid support;
 - (iv) a hybridization buffer, or components necessary for producing said buffer;
 - (v) a wash solution, or components necessary for producing said solution;
 - (vi) when appropriate, a means for detecting the hybrids resulting from the preceding hybridization.